Components of the Spore Wall of *Pithomyces chartarum*

The cell walls of fungi are known to contain large quantities of carbohydrate material, mainly composed of polymers of glucose, galactose, mannose and amino-sugars[1,2], but only in a few cases have the components been isolated from the walls and characterized. It is generally accepted that glycoproteins account for a considerable portion of the wall material in yeast[3], as they do in the hyphal walls of the filamentous fungus, *Pithomyces chartarum*[4]. Very little is known about the composition of spore walls; those of *Aspergillus oryzae* contain carbohydrate, protein and nucleic acid[5], and those of *Mucor rouxii* have carbohydrate, protein, lipid and pigment[6], the carbohydrate having been isolated as glucan, mannan and chitosan.

Spores, a gift from Dr. A. Smith, Ruakura Animal Research Station, New Zealand, were broken by disruption with glass beads in a Braun disintegrator (Shandon, Ltd., London), freed from cytoplasmic contamination by repeated sedimentation in 0·1 per cent sodium chloride and isolated after exhaustive dialysis followed by freeze-drying. The black pigmented product was shown to contain 45 per cent carbohydrate, 3·6 per cent bound hexosamine and 18·8 per cent protein (Table 1). Treatment with 10 per cent trichloroacetic acid failed to extract any material from the isolated walls (in contrast 15 per cent of the hyphal wall of the same organism was solubilized as phosphoglycoprotein under the same conditions[4]). Isolation of the cell wall components was achieved by extraction with anhydrous ethylenediamine at 37° C. On removal of the solvent, two chemically distinct fractions were obtained (Table 1), one soluble in ethylenediamine and soluble in water, *SWA* (4 per cent), the other soluble in ethylenediamine but insoluble in water, *SWB* (16 per cent). A residue, insoluble in ethylenediamine, was also recovered, *SWR*. Each fraction was shown to contain carbohydrate and protein. In addition the final residue contained all the wall pigment. The nature of the carbohydrate portion of each fraction was shown by examination of the acid hydrolysates using thin-layer chromatography. Each fraction contained glucose and glucosamine; in addition, *SWA* contained galactose and mannose. Ion-exchange chromatography of the acid hydrolysates of the three fractions showed the presence of fifteen amino-acids, in variable amounts, namely, asp, glu, ser, gly, ala, leu, ile, pro, val, lys, arg, thr, phe, his, met. Electrophoresis of the two extracted fractions on glass-fibre paper using formic–acetic acid buffer, 0·05 M, *p*H 2·0, indicated co-migration of the carbohydrate and protein in each case. In addition, *SWA* was chromatographed on a DEAE-cellulose column. Two fractions were obtained, one of which was not absorbed in *tris*-hydrochloric acid buffer, 0·001 M, *p*H 8·1, the other being eluted with the same buffer, 0·05 M, *p*H 8·1. The two glycoprotein fractions which were isolated from the spore walls were found to contain the same sugar and amino-acid components as the corresponding glycoproteins similarly extracted from the hyphal walls of the same organisms—the main difference being that the spore wall components contained less carbohydrate and more protein than the hyphal wall fractions. No evidence was found for the presence of the highly phosphorylated type of glycoprotein existing in the hyphal wall[4].

Table 1. Composition of Spore Wall Fractions

	Spore wall	*SWA*	*SWB*	*SWR*
Carbohydrate (per cent)	45 (40)†	50 (70)	61 (64)	43 (70)
Nitrogen (per cent)	3·3 (4·5)	4·3 (3·8)	4·0 (4·2)	3·1 (2·5)
Hexosamine[8] (per cent)	3·6 (10)	0·66 (1·9)	0·35 (4·6)	3·1 (10)
Protein (difference)	17·5 (25)	26·6 (22·8)	24·9 (23·8)	17·7 (10)
Glucose*	+	+	+	+
Galactose	+	+		
Mannose	+	+		
Amino-acids	+	+	+	+

* +, Present; −, absent or trace in acid hydrolysates.
† Figures in brackets are values for corresponding hyphal wall fractions, namely, whole wall, 2*A*, 2*B* and residue.

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1 Crook, E. M., and Johnson, I., *Biochem. J.*, **83**, 325 (1962).
2 Russell, D. W., Sturgeon, R. J., and Ward, V., *J. Gen. Microbiol.*, **36**, 289 (1964).
3 Korn, E., and Northcote, D. H., *Biochem. J.*, **75**, 12 (1960).
4 Sturgeon, R. J., *Biochem. J.*, **92**, 60 P (1964).
5 Horikoshi, K., and Iida, S., *Biochim. Biophys. Acta*, **83**, 197 (1964).
6 Bartnicki-Garcia, S., and Reyes, E., *Arch. Biochem. Biophys.*, **108**, 125 (1964).
7 Francois, C., Marshall, R. D., and Neuberger, A., *Biochem. J.*, **83**, 335 (1962).
8 Cessi, C., and Pileigo, F., *Biochem. J.*, **77**, 508 (1960).

PHYSIOLOGY

Reflex Activation of the Soleus Muscle of the Decerebrate Cat by Vibration

The primary afferent ending of the mammalian muscle spindle is highly sensitive to vibration[1–4]. In the presence of fusimotor activity it may discharge an impulse on each cycle of vibration applied to the muscle tendon, for vibrations of frequencies up to 300–400/sec and of amplitudes of a fraction of a mm (refs. 2 and 4). In the decerebrate cat, maintained stretch of an extensor muscle usually evokes from it a prolonged reflex contraction—the classical stretch reflex[5]. This reflex is generally believed to depend on the excitation of the primary endings of the spindle by the stretching of the muscle. Thus, it might be anticipated that vibration would also evoke a 'stretch reflex' in the decerebrate cat. The present experiments show that it does, indeed, do so.

The experiments were carried out on cats decerebrated under deep ether anaesthesia by transecting the mid-brain between the colliculi. The soleus muscle was investigated and the technique was generally similar to that described earlier[6]. The vibration was applied to the muscle tendon by connecting it to a vibrator (Pye-Ling, *V* 50), the steady current through which was modulated sinusoidally. The amplitude of vibration was determined by microscopic observation using a micrometer eyepiece. The contraction of soleus was recorded by connecting its tendon to an isometric myograph which was mounted on the end of the vibrator and through which the vibration was transmitted. The myograph utilized a semi-conductor strain gauge. Its maximum frequency of response was deliberately restricted to about 25/sec by electrical low-pass filters. This served to remove from its output the tension changes resulting directly from the vibration. The electrical activity of soleus was recorded from a pair of wires placed on its belly about 3 cm apart; virtually all the nerves in the leg, thigh and hip regions were severed except for that to soleus, so there was little doubt that the recorded activity did arise from soleus[6]. The leg was held by drills through the distal ends of the tibia and of the femur. The summed compliance of this fixation and that of the moving element of the vibrator was about 1 mm/kg; this comparative lack of rigidity is immaterial for the demonstration of the present effects.

Fig. 1 shows the typical reflex response of soleus to vibration. The initial length of the muscle was a few mm short of the maximum extension possible physiologically, but in this case there was no appreciable maintained tonic stretch reflex; the initial tension was 80 g wt. Vibration of 10μ amplitude at 200/sec elicited a contraction of 100 g wt. accompanied by appreciable electrical activity of the muscle. 100μ amplitude vibration caused a contraction of 500 g wt., accompanied by a massive